



<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 26666U

<150> KR 2002-55575

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<160> 19

<170> KopatentIn 1.71

<210> 1

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 1

<400> 1

ggctcttcag ccactccttt ggtgaag

27

<210> 2

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 2



<400> 2  
gcggatcctc agggggtgac gat

23

<210> 3  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> CALB primer 3

<400> 3  
gcggatccgg gggtgacgat gccggag

27

<210> 4  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> GPD-err primer

<400> 4  
gcagagctaa ccaataagg

19

<210> 5  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>



<223> T-0 primer

<400> 5

tgcagttgaa cacaaccac

19

<210> 6

<211> 1023

<212> DNA

<213> Candida antarctica

<220>

<221> sig\_peptide

<222> (1)..(51)

<223> secretion signal

<400> 6

atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtag cgccactccc	60
ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc	120
gatgcggggtc tgacctgcca ggggtgcttcg ccattcctcg tctccaaacc catccttctc	180
gtccccggaa cgggcaccac aggtccacag tcgttcgact cgaactggat ccccctctct	240
gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc	300
caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac	360
aacaagcttc ccgtgctcac ctgggtcccag ggtggtctgg ttgcacagtg ggggtctgacc	420
ttcttcccca gtatcaggtc caaggatgat cgacttatgg cctttgcgcc cgactacaag	480
ggcacctgcc tcgcccggcc tctcgatgca ctgcgggtta gtgcaccctc cgtatggcag	540



caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtgggtct gacccagatc	600
gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac	660
tcgccactcg actcatccta cctcttcaac gggaagaacg tccaggcaca ggctgtgtgt	720
gggccgctgt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc	780
ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacc	840
gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggctgc cgcggtgcg	900
ctcccggcgc cggcggtgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac	960
ctcatgccct acgcccgcc ctttgagta ggcaaaagga cctgctccg catcgtcacc	1020
ccc	1023

<210> 7  
 <211> 1023  
 <212> DNA  
 <213> Candida antarctica

<220>  
 <221> sig\_peptide  
 <222> (1)..(51)  
 <223> secretion signal

<400> 7	
atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtag cgccactcct	60
ttggtgaago gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc	120



gatgcggggtc tgacctgcca aggtgcttcg ccatacctcg tctccaaacc catccttctc	180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct	240
gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc	300
caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac	360
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggctctgacc	420
ttcttcccca gtatcaggtc caaggctgat cgacttatgg cctttgcgcc cgactacaag	480
ggcaccgtcc tcgccggccc tctcgatgca ctgcgggtta gtgcaccctc cgtatggcag	540
caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gaccagatc	600
gtgcccacca ccaacctcta ctcggcgacc gaagagatcg ttcagcctca ggtgtccaac	660
tcgccactcg actcactcta ccttttcaac ggaaagaacg tccaggcaca ggctgtgtgt	720
gggccgcagt tcgtcatcga ccatgcaggc tcgtcacct cgcagttctc ctacgtcgtc	780
ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcggacta tggcattacg	840
gactgcaacc ctcttccgc caatgatctg actcccgagc aaaaggctgc cgcggctgcg	900
ctcccggcgc cggcggctgc agccatctg gcgggtccaa agcagaactg cgagcccgc	960
ctcatgccct acgcccgc ctttgagta ggcaaaagga cctgctccgg catcgtcacc	1020
ccc	1023

<210> 8  
 <211> 1023  
 <212> DNA



<213> Candida antarctica

<220>

<221> sig\_peptide

<222> (1)..(51)

<223> secretion signal

<400> 8

atgaatatat ttacatatt ttgtttttg ctgtcattcg ttcaaggtac cgccactcct	60
ttggtgaagc gtctgccttc cggttcggac cctgcctttt cgcagcccaa gtcggtgctc	120
gatgcgggtc tgacctgcca gggtgcttcg ccacctcgg tctccaaacc catccttctc	180
gtccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct	240
ggcagctgg gttacacacc ctgctggatc tcaccccgcc cgttcatgct caacgacacc	300
caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac	360
aacaagcttc ccgtgctcac ctggtcccag ggtggtctgg ttgcacagtg gggctctgacc	420
ttcttcccca gtatcagggtc caaggctgat cgacttatgg cctttgccc cgactacaag	480
ggcaccgtcc tcgccggccc tctcgatgca ctgcggtta gtgcaccctc cgtatggcag	540
caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gaccagatc	600
gtgcccacca ccaacctcta ctggcgacc gacgagatcg ttcagcctca ggtgtccaac	660
tcgccactcg actcatccta cctcttcaac ggaaagaacg tccaggcaca ggctgtgtgt	720
gggccgcagt tcgtcatcga ccatgcaggc tcgctcacct cgcagttctc ctacgtcgtc	780
ggtcgatccg cctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacg	840



gactgcaacc ctcttcccg c caatgatctg actcccgagc aaaaggtcgc cgcgggtgcg 900

ctcctggcgc cggcgggtgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 960

ctcatgccct acgcccggcc ctttgca gta ggcaaaagga cctgctccgg catcgtcacc 1020

ccc 1023

<210> 9

<211> 343

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (1)..(17)

<223> secretion signal

<400> 9

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr

50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

65 70 75 80



Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270



Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro Gly Ser  
340

<210> 10

<211> 343

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (1)..(17)

<223> secretion signal

<400> 10

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly



35	40	45
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr		
50	55	60
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser		
65	70	75
		80
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met		
	85	90
		95
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr		
100	105	110
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp		
115	120	125
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser		
130	135	140
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys		
145	150	155
		160
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro		
165	170	175
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg		
180	185	190
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser		
195	200	205
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp		
210	215	220
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys		



225	230	235	240
Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe			
	245	250	255
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala			
	260	265	270
Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn			
	275	280	285
Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro			
	290	295	300
Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp			
305	310	315	320
Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser			
	325	330	335
Gly Ile Val Thr Pro Gly Ser			
	340		

<210> 11  
 <211> 341  
 <212> PRT  
 <213> Candida antarctica

<220>  
 <221> SIGNAL  
 <222> (1)..(24)  
 <223> secretion signal

<400> 11



Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190



Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro  
340

<210> 12

<211> 26

<212> DNA

<213> Artificial Sequence



<220>

<223> CALB primer 4

<400> 12

ctcatatgct accttccggt tcggac

26

<210> 13

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> a-amylase secretion signal

<400> 13

Met Met Val Ala Trp Trp Ser Leu Phe Leu Tyr Gly Leu Gln Val Ala

1

5

10

15

Ala Pro Ala Leu Ala

20

<210> 14

<211> 317

<212> PRT

<213> Candida antarctica

<400> 14

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu

1

5

10

15

Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys

20

25

30



Pro	Ile	Leu	Leu	Val	Pro	Gly	Thr	Gly	Thr	Thr	Gly	Pro	Gln	Ser	Phe
		35				40					45				
Asp	Ser	Asn	Trp	Ile	Pro	Leu	Ser	Ala	Gln	Leu	Gly	Tyr	Thr	Pro	Cys
		50				55					60				
Trp	Ile	Ser	Pro	Pro	Pro	Phe	Met	Leu	Asn	Asp	Thr	Gln	Val	Asn	Thr
	65				70				75				80		
Glu	Tyr	Met	Val	Asn	Ala	Ile	Thr	Thr	Leu	Tyr	Ala	Gly	Ser	Gly	Asn
			85					90					95		
Asn	Lys	Leu	Pro	Val	Leu	Thr	Trp	Ser	Gln	Gly	Gly	Leu	Val	Ala	Gln
		100					105					110			
Trp	Gly	Leu	Thr	Phe	Phe	Pro	Ser	Ile	Arg	Ser	Lys	Val	Asp	Arg	Leu
	115					120					125				
Met	Ala	Phe	Ala	Pro	Asp	Tyr	Lys	Gly	Thr	Val	Leu	Ala	Gly	Pro	Leu
	130					135					140				
Asp	Ala	Leu	Ala	Val	Ser	Ala	Pro	Ser	Val	Trp	Gln	Gln	Thr	Thr	Gly
	145				150					155			160		
Ser	Ala	Leu	Thr	Thr	Ala	Leu	Arg	Asn	Ala	Gly	Gly	Leu	Thr	Gln	Ile
		165					170					175			
Val	Pro	Thr	Thr	Asn	Leu	Tyr	Ser	Ala	Thr	Asp	Glu	Ile	Val	Gln	Pro
		180					185					190			
Gln	Val	Ser	Asn	Ser	Pro	Leu	Asp	Ser	Ser	Tyr	Leu	Phe	Asn	Gly	Lys
		195				200				205					
Asn	Val	Gln	Ala	Gln	Ala	Val	Cys	Gly	Pro	Leu	Phe	Val	Ile	Asp	His
	210					215				220					



Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala  
225                      230                      235                      240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr  
                    245                      250                      255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val  
                    260                      265                      270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly  
                    275                      280                      285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe  
                    290                      295                      300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro  
305                      310                      315

<210>    15  
<211>    28  
<212>    DNA  
<213>    Artificial Sequence

<220>  
<223>    LQ53 primer

<400>    15  
gctgtgtgtg ggccgcagtt cgtcatcg

28

<210>    16  
<211>    30  
<212>    DNA



<213> Artificial Sequence

<220>

<223> LQ35 primer

<400> 16

gcatggtcga tgacgaactg cggccacac

30

<210> 17

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LP53 primer

<400> 17

gtcgccgcgg ctgcgctccc ggcgccggcg

30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

<400> 18

ctgcagccgc cggcgccggg agcgagcc

29



<210> 19  
<211> 343  
<212> PRT  
<213> Candida antarctica

<400> 19  
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly  
1 5 10 15  
Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala  
20 25 30  
Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly  
35 40 45  
Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr  
50 55 60  
Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser  
65 70 75 80  
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met  
85 90 95  
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr  
100 105 110  
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp  
115 120 125  
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser  
130 135 140  
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys  
145 150 155 160



Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro  
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg  
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser  
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp  
210 215 220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys  
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe  
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala  
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn  
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro  
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp  
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser  
325 330 335

Gly Ile Val Thr Pro Gly Ser  
340